AN ACID PROTEASE FROM AURICULARIA AURICULA-JUDAE (Fr.) Quel

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AN ACID PROTEASE FROM *AURICULARIA AURICULA-JUDAE* (Fr.) Quél

By

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Abstract

The acid protease was partially purified from *Auricularia auricula-judae* (Fr.) Quél. The maximum activity was found in the acidic pH region using cyanogen bromide treated hen lysozyme as a substrate. It was found to be 4.7. The optimum temperature using casein is 50°C at pH 6.0. The protease was not influenced by EDTA, phenylmethanesulfonyl fluoride and iodoacetamide.

Introduction

In a screening test for fungus with proteolytic activity toward milk casein we found a strain of *Auricularia auricula-judae* (Fr.) Quél. High proteolytic activity was observed in its extract at the start, but the activity decreased step by step in the process of isolation. Eventually, a stable acid protease was partially purified from all the ones that were active at the beginning.

Acid proteases catalyzed the hydrolysis of peptide bonds at acidic pH values. They occur widely in organisms and are important in many diverse biological functions.

We present here some properties of the acid protease of *Auriclaria auricula-judae*.

Experimental

Materials– Kikurage, *Auricularia auricula-judae*, "jew's ear" was obtained from a grocer in Kagoshima Prefecture.

DEAE-Cellulose DE 23 was a product of Whatman Ltd. Bio-Gel P-10 was a product of BIO-RAD laboratories. Phenylmethanesulfonyl fluoride was purchased from Wako Pure Chemical Industries, Ltd. Casein was a product of E. Merck. Iodoacetamide was purchased from Nakarai Chemicals Ltd.

Assay of Protease– Proteolytic activity was measured by the method of Kunitz*(1)*, with milk casein as a substrate. One ml of enzyme solution was added to 1 ml of a solution of 2%

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(w/w) casein containing M/30 phosphate buffer, pH 7.0, at 30°C. After incubation for 60 min the reaction was stopped by the addition of 3 ml of 5% trichloroacetic acid. After standing for 30 min at room temperature, the precipitate was removed by filtration through Toyo filter paper No. 5C and the absorbancy at 280 nm of the trichloroacetic acid-soluble peptides formed was determined with a Shimadzu QV-50 spectrophotometer.

A unit of activity was defined as that amount which yielded 0.001 A_{280nm} unit of change per minute under the conditions mentioned above.

Concerning the experiment checking for the effect of varying the pH on the proteolytic activity, cyanogen bromide treated hen egg white lysozyme as a substrate was used instead of milk casein for the reason stated later in this paper.

Results and Discussion

Isolation of Protease– When dried *Auricularia auricula-judae* was steeped in distilled water, its weight increased about three or four times. The swelled material was homogenized in a domestic mixer. The homogenate was packed into a cotton bag and centrifuged in a domestic spin drier. The extract obtained is colored light brown. Activated DEAE-cellulose fibers were added to the extract (70g wet weight per liter of extract). The mixture was stirred for 1 hr, at pH 7.0. The DEAE-cellulose fibers were collected by filtration through a cotton bag. The protease was eluted from the DEAE-cellulose fibers with 1.0 M NaCl. The elute was dialyzed against distilled water overnight. The dialysate was centrifuged to remove insoluble materials and placed on a colum of DEAE-cellulose DE 23(4.4 × 50cm) equilibrated with 0.02 M phosphate buffer, pH 7.0, containing 0.2 M NaCl. The column was eluted with a linear gradient going from 0.02 M phosphate buffer, pH 7.0 containing 0.2 M NaCl to 0.1 M phosphate buffer, pH 7.0 containing 1.5 M NaCl.

The chromatographic pattern is shown in Fig. 1. The active protein fraction were collected and precipitated by the addition of solid ammonium sulfate to 50% saturation. An aliquot of the precipitated enzyme was dissolved in distilled water and applied to a Bio-Gel P-10 column(2.7 × 96cm). The column was developed with distilled water. The active fraction was collected (Fig. 2) and used for subjecting to experiments. When 0.2 M NaCl solution was used as a eluent instead of the above distilled water, the colored peak became broad, therefore, we could not get as good separation as we did with water.

Effect of pH on the Enzymic Activity– The pH activity curve of the protease for hydrolysis of cyanogen bromide treated hen egg white lysozyme as a substrate showed optimal activity at pH 4.7 as shown in Fig. 3. It was not possible to perform the assay with casein in the above acidic pH range, because casein tends to precipitate below pH 6.0.

Effect of the Temperature on the Enzymic Activity– The enzyme exhibited maximum activity at around 50°C, at pH 6.0 as shown in Fig. 4.

Effect of Various Compounds on the Enzymic Activity– Iodoacetamide, EDTA, and phenylmethanesulfonyl fluoride showed no effects on the activity of the protease. From these behaviors and the acidic pH optimum of this enzyme, it seems that the protease of

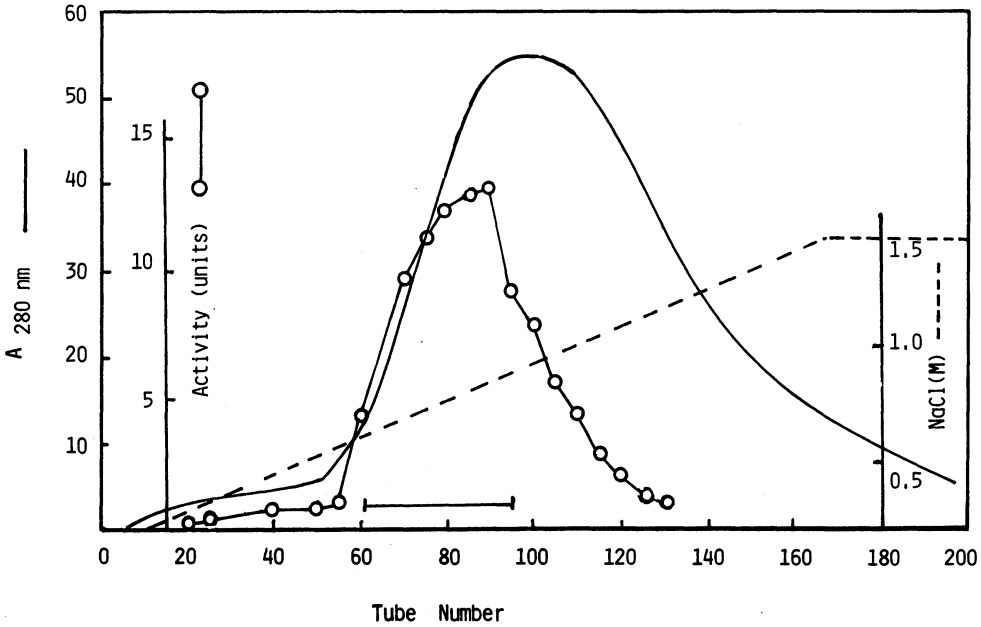

Fig. 1. Chromatography of extract of *Auricularia auricula-judae* on DEAE-cellulose.
The extract was chromatographed on a DEAE-cellulose DE 23 column(4.4 × 50cm). The column was eluted with a linear gradient from 0.02 M phosphate buffer, pH 7.0, containing 0.2 M NaCl to 0.1 M phosphate buffer, pH 7.0 containing 1.5 M NaCl. Fifteen ml of fractions were collected. An aliquot(0.5 ml) of each tube were added to 0.5 ml of 0.02 M phosphate buffer, pH 7.0 and then the proteolytic activity was assayed.

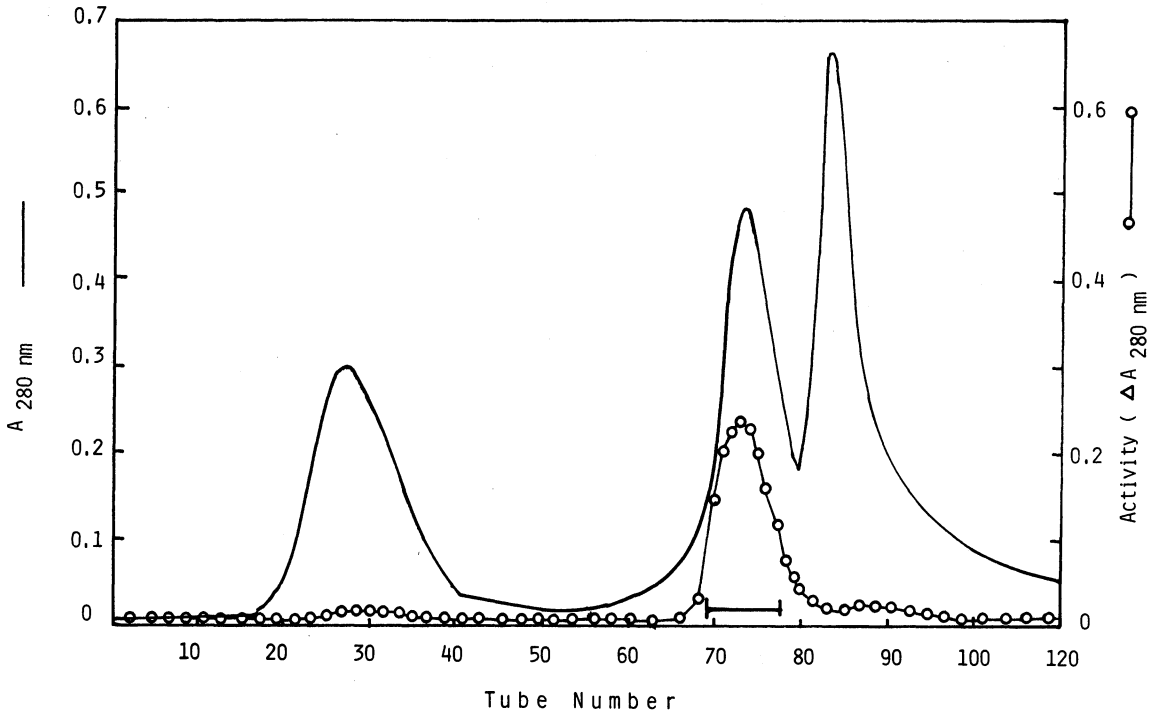

Fig. 2. Gel filtration of the protease fraction on Bio-Gel P-10.
The preparation obtained from the DEAE-cellulose DE 23 chromatography purification step was applied to a column(2.7 × 96cm) on Bio-Gel P-10. The column was developed with distilled water. Six ml of fractions were collected. The proteolytic activity was measured by the same method as described in Fig. 1.

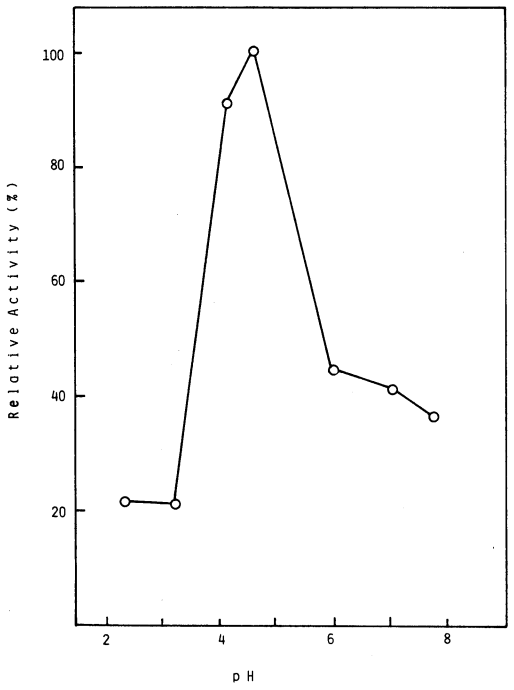

Fig. 3. Effect of pH on the proteolytic activity.
The incubation mixture consisted in 1.0 ml of enzyme solution and 1.0 ml of 0.5% cyanogenbromide treated hen egg white lysozyme as a substrate in 0.2 M phosphate-0.1 M citric acid buffer at various pH values.

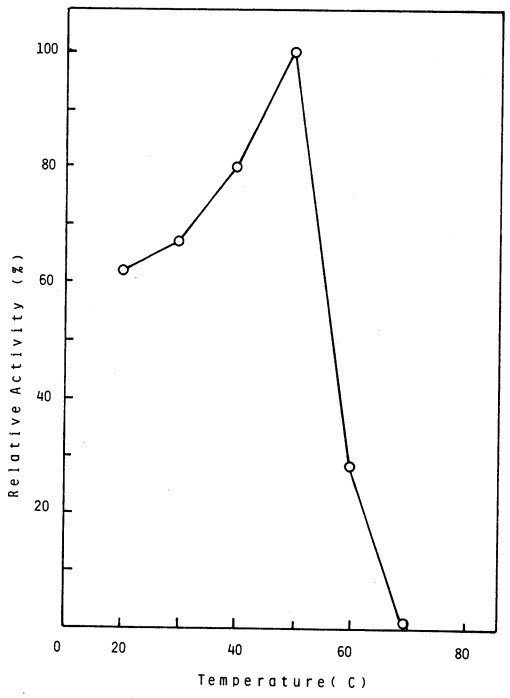

Fig. 4 Effect of temperature on the proteolytic activity.
The incubation mixture consisted in 1.0 ml of enzyme in 0.02 M phosphate buffer, pH 7.0 and 1.0 ml of 1% casein solution. The assay solution was incubated at various temperature for 30 min.

Table 1. Effect of various compounds on the proteolytic activity.
The enzyme was preincubated in 1.0 ml of 0.05 M phosphate buffer, pH 7.0, containing various compounds for 60 min at 30°. After incubation, 1.0 ml of 1% casein was added to the mixture and the activity was assayed by the standard procedure.

Addition	Concentration[a] (m M)	Relative activity[b] (%)
None		100
EDTA	0.5	99
PMSF[c]	0.5	97
ICH_2CONH_2	0.5	95

a Concentrations are those in the preincubation mixture.
b Activity of a control with no addition was taken as 100%.
c Phenylmethanesulfonyl fluoride.

Auricularia auricula-judae is classified into carboxyl protease.

Reference

(1) Kunitz, M. (1947) J. Gen. Physiol. **30**, 291–310.